Distribution and Diversity of Russian Wheat Aphid (Hemiptera: Aphididae) Biotypes in North America

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ABSTRACT Wheat, *Triticum aestivum* L., with Russian wheat aphid, *Diuraphis noxia* (Kurdjumov) (Hemiptera: Aphididae) resistance based on the *Dn4* gene has been important in managing Russian wheat aphid since 1994. Recently, five biotypes (RWA1–RWA5) of this aphid have been described based on their ability to differentially damage RWA resistance genes in wheat. RWA2, RWA4, and RWA5 are of great concern because they can kill wheat with *Dn4* resistance. In 2005, 365 Russian wheat aphid clone colonies were made from collections taken from 98 fields of wheat or barley, *Hordeum vulgare* L., in Oklahoma, Texas, New Mexico, Colorado, Kansas, Nebraska, and Wyoming to determine their biotypic status. The biotype of each clone was determined through its ability to differentially damage two resistant and two susceptible wheat entries in two phases of screening. The first phase determined the damage responses of Russian wheat aphid wheat entries with resistance genes *Dn4*, *Dn7*, and susceptible 'Custer' to infestations by each clone to identify RWA1 to RWA4. The second phase used the responses of Custer and 'Yuma' wheat to identify RWA1 and RWA5. Only two biotypes, RWA1 and RWA2, were identified in this study. The biotype composition across all collection sites was 27.2% RWA1 and 72.8% RWA2. RWA biotype frequency by state indicated that RWA2 was the predominant biotype and composed 73–95% of the biotype complex in Texas, Oklahoma, Colorado, and Wyoming. Our study indicated that RWA2 is widely distributed and that it has rapidly dominated the biotype complex in wheat and barley within its primary range from Texas to Wyoming. Wheat with the *Dn4* resistance gene will have little value in managing RWA in the United States, based on the predominance of RWA2.

KEY WORDS host plant resistance, barley, wheat, host race

The Russian wheat aphid, *Diuraphis noxia* (Kurdjumov) (Hemiptera: Aphididae), has been a significant pest of barley, *Hordeum vulgare* L., and winter wheat, *Triticum aestivum* L., in the western half of the United States since its introduction in 1986. Chemical control was the primary means of managing this pest until Russian wheat aphid-resistant wheat cultivars with the *Dn4* resistance gene were deployed in 1994 (Quick et al. 1996). Resistant wheat cultivars were an economical solution to the Russian wheat aphid problem for almost a decade.

Biotypic variation in Russian wheat aphid to plant resistance has been documented within populations in France and former Russia (Puterka et al. 1992) and between populations from different countries (Puterka et al. 1992, Baskey 2002, Smith et al. 2004). In 2003, a new biotype was discovered that was capable of damaging Russian wheat aphid-resistant wheat cultivars with resistance imparted by the *Dn4* resistance gene (Haley et al. 2004). Another recent study discovered three other Russian wheat aphid biotypes with unique biotype profiles when screened against the nine designated Russian wheat aphid resistance genes in wheat, *Dn1* to *Dn9* (Burd et al. 2006). A system for naming the Russian wheat aphid biotypes has been proposed (Burd et al. 2006) where designations of RWA1 was used for the Russian wheat aphid population originally collected from Bailey Co., TX, in 1986 (Burd et al. 1993). RWA2 classification was used for the Colorado biotype that damages *Dn4* resistance in wheat, and RWA3, RWA4, and RWA5 designated those biotypes collected in Texas and Wyoming that differentially damage *Dn1*–*Dn9* resistance in wheat (Burd et al. 2006). Research has determined (Puterka et al. 2006) that the five Russian wheat aphid biotypes (RWA1 to RWA5) do not severely damage the primary sources of resistance in barley, STARS 9301B (Mornhinweg et al. 1995) and STARS 9577B (Mornhinweg et al. 1999). The recent appearance of these new biotypes makes it critical to determine the extent of their frequency and distribution in the United States to successfully deploy Russian wheat aphid resistance in wheat.

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Table 1. Response of the nine primary resistance genes in wheat to the Russian wheat aphid

Biotype	Resistance gene/cultivar response										
	Dn1	*Dn2*	*Dn3*	*Dn4**	*Dn5*	*dn6*	*Dn7**	*Dn8*	*Dn9*	Yuma*	Custer*
RWA1	S	R	R	R	R	R	R	S	S	S	S
RWA2	S	S	S	S	S	S	R	S	S	S	S
RWA3	S	S	S	S	S	S	S	S	S	S	S
RWA4	S	S	S	R	S	R	S	S	S	S	S
RWA5	S	S	S	R	R	R	R	S	S	R	S

Two additional wheat varieties are needed to differentiate biotypes RWA1 from RWA5. The four plant entries followed by an asterisk were used to identify biotypes RWA1–RWA5.

The objective of this study was to determine the distribution and diversity of Russian wheat aphid biotypes within populations that reside in the hard winter wheat and spring malting barley regions of the United States east of the Rocky Mountains.

Materials and Methods

Russian wheat aphid samples were collected from Oklahoma, Texas, New Mexico, Colorado, Kansas, Nebraska, and Wyoming in 2005. The main wheat and barley production areas within the common distribution of the Russian wheat aphid (Burd et al. 1998) were sampled. Collection sites were selected off primary or secondary roads that transected major wheat or barley production areas of each state. Sites were 8–40 km apart and distance depended on the continuity of the wheat and barley fields. We collected 3–20 infested tillers per site, depending on infestation level, and we placed the tillers in petri dishes that contained wet filter paper and stored them in an icebox for transportation to the laboratory. One individual aphid from each tiller was transferred to 'Custer' wheat and caged to produce a clone colony in a growth room with a temperature of 25°C and a photoperiod of 14:10 (L:D) h.

The biotype of each clone was determined by screening its feeding damage on two resistant and two susceptible wheat entries in two phases. In the first phase, each clone was characterized on Russian wheat aphid-susceptible Custer wheat, 'Yumar' with the *Dn4* gene, and 94M370 with the *Dn7* gene, to identify RWA1 through RWA4 (Table 1). The *Dn4* gene imparts resistance in Yumar to RWA1, RWA4, and RWA5. The *Dn7* gene confers resistance to the RWA1, RWA2, and RWA5. After this study was initiated, biotypes RWA3 through RWA5 were verified (Burd et al. 2006). In the first phase of screening, RWA1 and RWA5 would have given the same response for all the plant entries (Custer, Yumar, and 94M370). Therefore, the colonies underwent a second phase of screening where the differential responses of Custer and Yuma wheat were used to discern RWA1 from RWA5 (Table 1).

The three plant entries for the first phase of screening were planted in fine sand in 200-ml foam coffee cups. Two seeds per plant entry were planted with the three plant entries arranged in a triangular pattern separated by a distance of 2.5 cm. Plant entries were marked by colored plastic stakes (1 cm in width × 10 cm in length) placed in the center of the triangular planting pattern. Plants were thinned to one plant per entry when the plants reached 2–3 cm in height. The infested plants were caged and held in a growth chamber with a temperature of 25°C and a photoperiod of 14:10 (L:D) h. Three weeks after infestation, plants were rated for leaf rolling on a 1–3 scale, where 1 is not rolled, 2 is folded, and 3 is fully rolled; and for leaf chlorosis on a 1–9 scale (Burd et al. 1993), where 1 is no damage/chlorosis, 2 is 1–5%, 3 is 6–20%, 4 is 21–35%, 5 is 35–50%, 6 is 51–65%, 7 is 66–80%, 8 is 81–95%, and 9 is 96–100% necrosis/chlorosis.

Comparisons in the susceptibility of Yuma and Custer to the Russian wheat aphid clones were made using a similar aforementioned cup design and screening procedure. However, the entries were evaluated when Custer rated a 7–8 for chlorosis/necrosis (2–3 wk postinfestation) to prevent the plants from being overwhelmed and killed by the aphid infestation before plants could be scored.

Russian wheat aphid biotypes were classified by using leaf chlorosis damage ratings for each plant entry where the plant was considered resistant (R) if the chlorosis rating was 1–5 and susceptible (S) if the chlorosis rating was 6–9 to be consistent with classifications by Haley et al. (2004) and Burd et al. (2006). Each clone was given a biotype designation based on the differential virulence profile to the *Dn4* and *Dn7* resistance genes in wheat and to Custer, and Yuma wheat (Table 1).

Biotype groups across all plant differentials for each screening phase were analyzed by a two-way (clone, plant entry) analysis of variance (ANOVA). Screening phases with significant ($P = 0.05$) clone-by-plant entry interactions had mean chlorosis ratings and leaf roll ratings for clones (biotypes) within each plant entry compared by Fisher's protected least significant difference (LSD) test ($P = 0.05$) (SAS Institute 2003).

Results and Discussion

There were 98 sample sites established in the seven states. All but 10 of the sites had 2–18 samples with the majority of sites having at least three samples taken and aphids successfully cloned. In total, 365 clones were established from these sites for biotype analysis (Fig. 1). Russian wheat aphid biotypes were based on the chlorotic damage responses to aphid feeding in

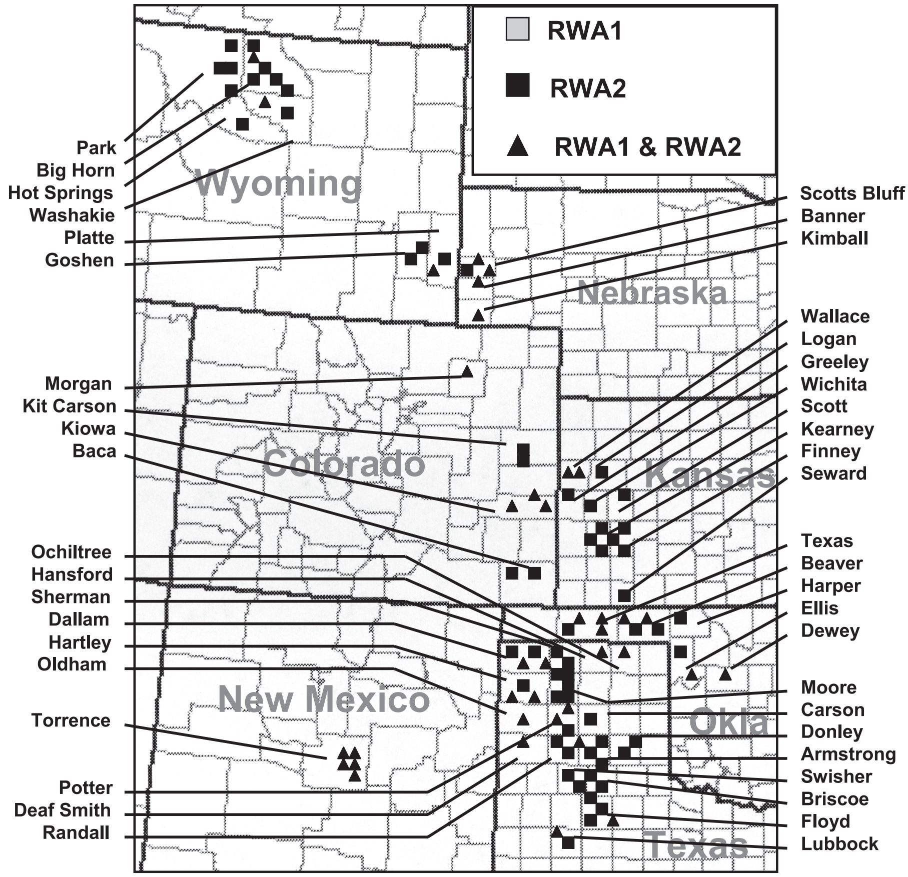

Fig. 1. Biotype composition for each site where Russian wheat aphid collections were made in 2005. Note that there were no sites where only RWA1 were collected; thus, there are no gray colored squares as referenced in the legend.

barley and wheat (Haley et al. 2004, Burd et al. 2006, Puterka et al. 2006). Screening the 365 clones against the plant entries resulted in identifying only two Russian wheat aphid biotypes, RWA1 and RWA2 (Table 2). In the first phase of screening, analysis of main effects for chlorosis indicated a significant clone (F = 16.6; df = 385, 4,537; P < 0.0001), plant entry (F = 4,0295.0; df = 2, 4,537; P < 0.0001), and clone-by-plant entry interaction (F = 16.0; df = 772, 4,537; P < 0.0001), suggesting that the plant entries were responding differently to the aphid clones. Analysis of main effects for leaf rolling (Table 3) reflected a similar relationship to chlorosis by having a significant clone (F = 4.6; df = 385, 4,537; P < 0.0001), plant entry (F = 9,942.0; df = 2, 4,537; P < 0.0001), and clone-by-plant entry interaction (F = 4.5; df = 772, 4,537; P < 0.0001). Infestations of RWA1 and RWA2 caused clear differential reactions in leaf chlorosis to resistant Yumar (*Dn4* gene) (Table 2). Leaf chlorosis ratings indicated Custer was susceptible, whereas 94M370 (*Dn7*) was resistant to both biotypes. Further support of resistance or susceptible ratings to biotypes RWA1 and RWA2 for the entries carrying *Dn4* and *Dn7* was observed in the leaf rolling response to Russian wheat aphid feeding (Table 3). Both sources of resistance did not roll in response to RWA1 feeding. Feeding by RWA2 induced leaf rolling in Yumar (*Dn4*) and produced no rolling response in 94M370 (*Dn7*) as re-

Table 2. Mean chlorosis rating for each plant differential in the first phase of screening 3 wk after infestation by each biotype

Biotype	Custer	Yumar (*Dn4*)	94M370 (*Dn7*)
RWA1	8.9 ± 0.01a	3.2 ± 0.04b	2.4 ± 0.03a
RWA2	9.0 ± 0.01a	7.9 ± 0.03a	2.5 ± 0.01a

Chlorosis rating: 1, no damage; 9, dead plant.
Means within columns followed by the same letter are not significantly different (P > 0.05; LSD).

Table 3. Mean leaf roll rating for each plant differential in the first phase of screening 3 wk after infestation by each biotype

Biotype	Custer	Yumar (*Dn4*)	94M370 (*Dn7*)
RWA1	3.0 ± 0.00a	1.5 ± 0.02b	1.2 ± 0.02a
RWA2	3.0 ± 0.00a	2.7 ± 0.03a	1.2 ± 0.01a

Roll rating: 1, flat leaf; 3, rolled leaf.
Means within columns followed by the same letter are not significantly different (P > 0.05; LSD).

Table 4. Mean chlorosis and leaf roll rating for each plant differential in the second phase of screening 2–2.5 wk after infestation by each biotype

Biotype	Chlorosis rating		Roll rating	
	Custer	Yuma	Custer	Yuma
RWA1	8.1 ± 0.14 NS	7.9 ± 0.01 NS	3.0 ± 0.02 NS	3.0 ± 0.10 NS
RWA2	7.9 ± 0.01	8.0 ± 0.01	3.0 ± 0.00	3.0 ± 0.00

Chlorosis rating: 1, no damage; 9, dead plant. Roll rating: 1, flat leaf; 3, rolled leaf.

The ANOVA resulted in no significant (NS) clone-by-plant entry interaction. Therefore, means within columns were not statistically compared.

ported in other studies (Haley et al. 2004, Burd et al. 2006).

The second phase of screening (Table 4) found no significant clone ($F = 2.5$; df = 1, 3,738; $P = 0.11$), plant entry ($F = 0.9$; df = 1, 3738; $P = 0.35$), or clone-by-plant entry interaction ($F = 3.0$; df = 1, 3,738; $P = 0.08$), indicating that Custer and Yuma responded equally to Russian wheat aphid feeding. Leaf chlorosis ratings and leaf roll ratings indicated that both wheat varieties were equally susceptible (Table 4).

The similar responses of these two cultivars (Table 1) to the 365 clones we tested indicated that there were only two biotypes present, RWA1 and RWA2.

Categorizing the aphid clones that were collected from the sites throughout the primary range of Russian wheat aphid from Texas to Wyoming determined that RWA2 was the predominate biotype (Fig. 1). Most of the samples within sites contained RWA2 only (■) and none of the sites produced only RWA1 (▢). Those states with sites that indicated a mixture of biotypes (▲) were present also showed RWA2 was present in high proportions. These states included Texas (66.7%), Oklahoma (70.8%), Colorado (68.2%), Wyoming (55.5%), Kansas (60.0%), and Nebraska (41%). The exception was the barley production area in New Mexico where all sites contained biotype mixtures but had a low percentage (22%) of RWA2.

Russian wheat aphid biotype distribution by state (Fig. 2) indicated that RWA2 was the predominant biotype and composed 75–93% of the biotype complex in Texas, Oklahoma, Colorado, and Wyoming. The only other Russian wheat aphid biotype we detected was RWA1, the original Russian wheat aphid biotype strain that was first reported in the United States in 1986. The biotype frequencies in Nebraska were nearly equal, whereas RWA1 still dominated the biotype complex in New Mexico. Greater than 90% of the samples from Wyoming and New Mexico were collected from spring barley; yet, the biotype complexes were considerably different between these states.

Russian wheat aphid biotype frequencies were also partitioned by region based on the presence of fairly continuous wheat or barley fields within a region and with each region interrupted by ≥150 km of arid grasslands; the Central Great Plains, Northern Great Plains, Big Horn Basin, and New Mexico High Plains (Fig. 3). This analysis indicated that the biotype complex was 82–90% RWA2 for all regions but the New Mexico High Plains (22%). The New Mexico study site

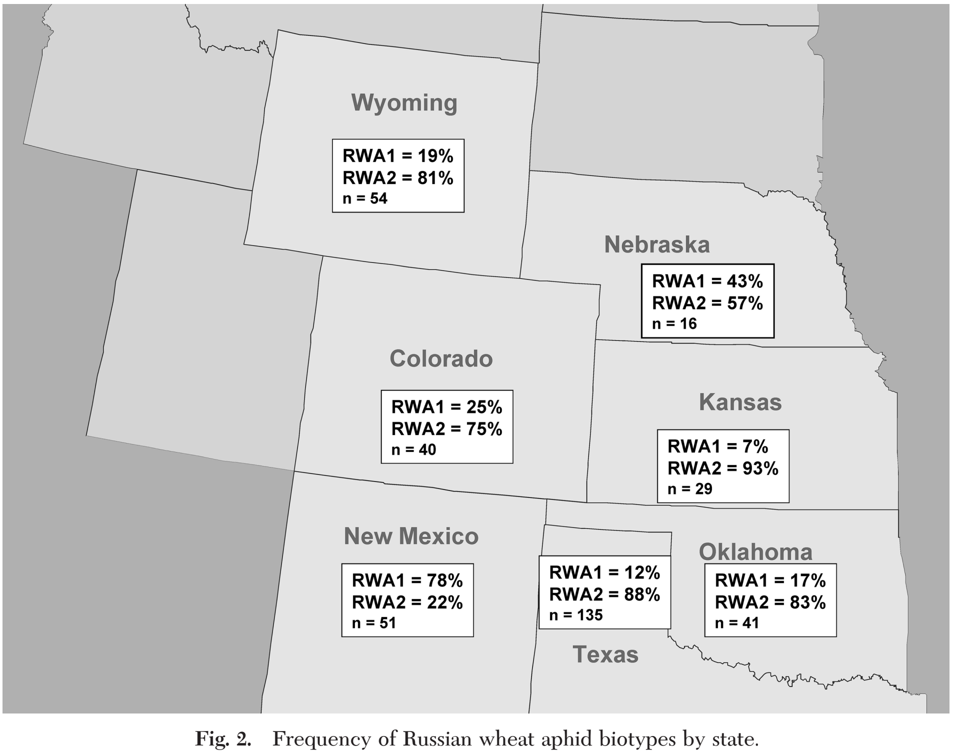

Fig. 2. Frequency of Russian wheat aphid biotypes by state.

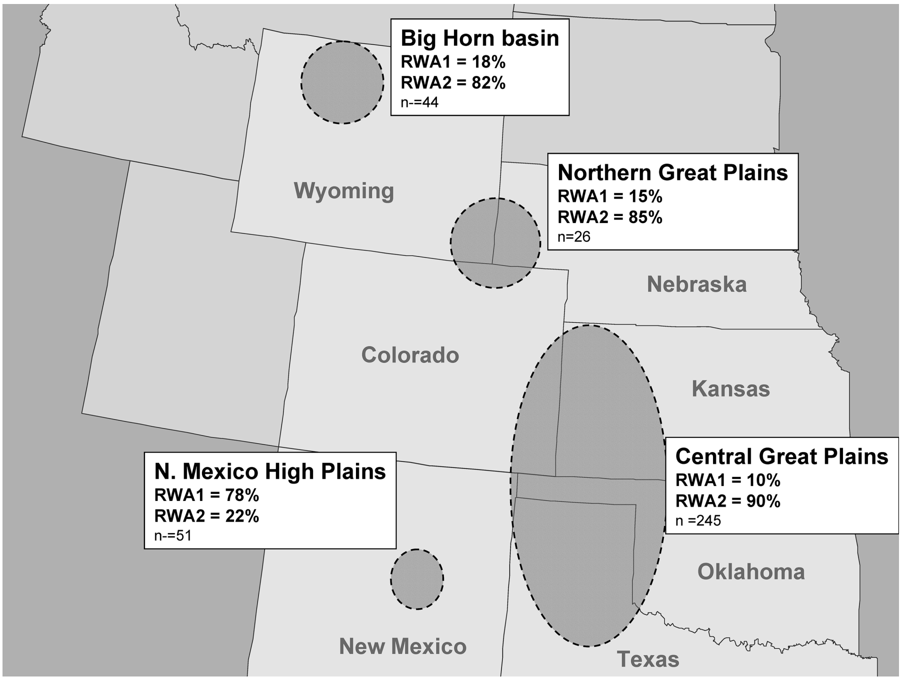

Fig. 3. Frequency of Russian wheat aphid biotypes by region.

represented a small barley production system with a large mountainous southwestern grassland ecosystem, which may have favored RWA1 fitness and survival over RWA2.

Burd et al. (2006) did not collect RWA2 outside of Colorado during their study, and they found only three unusual clones (RWA3, RWA4, and RWA5) out of 75 samples (4% new biotypes), during their assessment of biotypic diversity in Texas, Oklahoma, Nebraska, and Wyoming in 2002 and 2003. We did not detect RWA3–RWA5, despite that our sample areas had some overlap. These biotypes may have been too rare for our study to detect. However, two of the three new biotypes described by Burd et al. (2006) were virulent to Russian wheat aphid-resistant wheat with the *Dn4* gene; therefore, this aphid virulence was already present in Texas and Wyoming in 2002.

RWA1-resistant wheat based on *Dn*4 resistance has mainly been deployed in Colorado since 1994, and commercial plantings of RWA1-resistant wheat only made up 25% of the total wheat acreage in Colorado in winter 2003 and 2004 (Haley et al. 2004). Use of RWA1 resistance in states outside of Colorado where this aphid is not a persistent problem has been rare. The majority of wheat and barley acreage grown throughout the western United States is susceptible to Russian wheat aphid. Furthermore, a number of grasses also serve as important oversummering hosts and ecological reservoirs (Burd et al. 1998). Therefore, wheat is not essential for the survival of Russian wheat aphid in the United States. The predominance of RWA2 throughout the primary range of Russian wheat aphid indicates that its frequency was not directly influenced by the presence of RWA1-resistant wheat. There seems to be other ecological or biological factors that favor the presence of RWA2 over RWA1 besides the presence of *Dn4*-based Russian wheat aphid resistance in the field.

Our study did not identify a concentrated area of RWA2 that might have indicated a point of origin, based on the pattern of biotype distribution nationally, regionally, and by state. Apparently, RWA2 was well established before our study, and it was probably well established before its discovery in 2003. However, until it is determined how Russian wheat aphid biotypes occur, it cannot be assumed that RWA2 originated from a specific area and dispersed from that epicenter. It is possible that RWA2 could have arisen at multiple sites followed by local dispersion. The New Mexico sites had the lowest proportion of RWA2 in comparison with RWA1. Nevertheless, it seems that RWA2 has become firmly established throughout the primary range of Russian wheat aphid in the western half of United States. The extent and pervasiveness of RWA2 distribution are further exemplified by small collections ($n = 3$ clones each) of Russian wheat aphid near the Prosser, WA, area in 2004, and near Jackson, WY, in 2006, that both contained 33.3% RWA2 (G.J.P., unpublished data).

The dispersion of RWA1 throughout the hard red winter wheat area of the United States was rapid from the point of its first sighting in central Mexico in 1980 (Gilchrist et al. 1983) to its appearance in central Texas in 1986 (Stoetzel 1987). After Russian wheat aphid was discovered in the United States, it rapidly spread to the 17 small grain-producing states in the western half of the United States by 1987 (Morrison and Peairs 1998). Rapid dispersion seems to be characteristic of this aphid. Although RWA2 was first reported in Colorado in 2003 (Haley et al. 2004), we could not conclude that this area was the point of origin because of the extent of this biotypes distribution and lack of an association with the presence of Russian wheat aphid-resistant wheat. We can say that the dominance of RWA2 occurred rapidly, regardless of the mechanism that made its appearance possible, because Russian wheat aphid-resistant wheat with *Dn4* resistance gene was successfully managing Russian wheat aphid before the discovery of RWA2 in 2003. The extent of the distribution and predominance of RWA2 throughout the primary hard red winter wheat growing region of the western United States indicates that wheat with Russian what aphid resistance based on the *Dn4* gene will have little value in managing this pest. In contrast, the primary sources of RWA1 resistance in barley remain resistant to RWA2 to RWA5 (Puterka et al. 2006).

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